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L10 ANSWER 1 OF 9 MEDLINE
ACCESSION NUMBER: 2000044123 MEDLINE
DOCUMENT NUMBER: 20044123 PubMed ID: 10579522
TITLE: Directed evolution of an esterase: screening of enzyme libraries based on pH-indicators and a growth assay.
AUTHOR: Bornscheuer U T; Altenbuchner J; Meyer H H
CORPORATE SOURCE: Institute for Technical Biochemistry, University of Stuttgart, Germany.. bornscheuer@po.uni-stuttgart.de
SOURCE: BIOORGANIC AND MEDICINAL CHEMISTRY, (1999 Oct) 7 (10) 2169-73.
Journal code: 9413298. ISSN: 0968-0896.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991223

AB In order to resolve a sterically hindered 3-hydroxy ethyl ester, which was not accepted as substrate by 20 wild-type hydrolases, a directed evolution of an esterase from *Pseudomonas fluorescens* (PFE) was performed. Mutations were introduced using the *mutator* strain *Epicurian coli* XL1-Red. Enzyme libraries derived from seven mutation cycles were assayed on minimal media agar plates supplemented with pH indicators (neutral red and crystal violet), thus allowing the identification of active esterase variants by the formation of a red color caused by a pH decrease due to the released acid. A further selection criteria was introduced by using the corresponding glycerol ester, because release of the carbon source glycerol facilitates growth on minimal media. By this strategy, one double mutant (A209D and L181V) of PFE was identified, which hydrolyzed the 3-hydroxy ethyl ester in a stereoselective manner (25% ee for the remaining ester, E approximate to 5).

L10 ANSWER 2 OF 9 MEDLINE
ACCESSION NUMBER: 1999201088 MEDLINE
DOCUMENT NUMBER: 99201088 PubMed ID: 10099292
TITLE: Directed evolution of an esterase for the stereoselective resolution of a key intermediate in the synthesis of epothilones.
AUTHOR: Bornscheuer U T; Altenbuchner J; Meyer H H
CORPORATE SOURCE: Institute for Technical Biochemistry, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany.. itbubo@po.uni-stuttgart.de
SOURCE: BIOTECHNOLOGY AND BIOENGINEERING, (1998 Jun 5) 58 (5) 554-9.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 19990517
Last Updated on STN: 19990517

Entered Medline: 19990503

AB The directed evolution of an esterase from *Pseudomonas fluorescens* using the **mutator** strain *Epicurian coli* XL1-Red was investigated. Mutants were assayed for their ability to hydrolyze a sterically hindered 3-hydroxy ester, which can serve as a building block in the synthesis of epothilones. Screening was performed by plating esterase producing colonies derived from mutation cycles onto minimal media agar plates containing indicator substances (neutral red and crystal violet). Esterase-catalyzed hydrolysis of the 3-hydroxy ester (ethyl or **glycerol** ester) was detected by the formation of a red color due to a pH decrease caused by the released acid. Esterases isolated from positive clones were used in preparative biotransformations of the ethyl ester. One variant containing two mutations (A209D and L181V) stereoselectively hydrolyzed the ethyl ester resulting in 25% ee for the remaining ester.

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L10 ANSWER 3 OF 9 MEDLINE
ACCESSION NUMBER: 1998359889 MEDLINE
DOCUMENT NUMBER: 98359889 PubMed ID: 9693060
TITLE: Production and purification of the heavy-chain fragment C of botulinum neurotoxin, serotype B, expressed in the methylotrophic yeast *Pichia pastoris*.
AUTHOR: Potter K J; Bevins M A; Vassilieva E V; Chiruvolu V R; Smith T; Smith L A; Meagher M M
CORPORATE SOURCE: Department of Food Science and Technology, Biological Process Development Facility, University of Nebraska-Lincoln, 68583-0919, USA.
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1998 Aug) 13 (3) 357-65.
JOURNAL CODE: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980925
Last Updated on STN: 19980925
Entered Medline: 19980917

AB A recombinant Hc fragment of botulinum neurotoxin, serotype B (rBoNTB(Hc)), has been successfully expressed in a **Mut+** strain of the methylotrophic yeast *Pichia pastoris* for use as an antigen in a proposed human vaccine. The fermentation process consisted of batch phase on **glycerol**, followed by **glycerol** and methanol fed-batch phases yielding a final cell mass of 60 g/L (dcw) and was easily scaled-up to 60 L. A multistep ion-exchange chromatographic purification process was employed to produce 99% pure Hc fragment. The final yield of the purified antigen was 390 mg per kilogram of wet cell mass. The purified Hc fragment of serotype B was stable, elicited an immune response in mice, and protected upon challenge with native botulin.

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L10 ANSWER 4 OF 9 MEDLINE
ACCESSION NUMBER: 1998311078 MEDLINE
DOCUMENT NUMBER: 98311078 PubMed ID: 9648744
TITLE: Relocation of urf a from the mitochondrion to the nucleus

cures the mitochondrial mutator phenotype in the fission yeast *Schizosaccharomyces pombe*.

AUTHOR: Neu R; Goffart S; Wolf K; Schafer B

CORPORATE SOURCE: Institut fur Biologie IV (Mikrobiologie) der Rheinisch-Westfalischen Technischen Hochschule Aachen, Germany.

SOURCE: MOLECULAR AND GENERAL GENETICS, (1998 May) 258 (4) 389-96.
Journal code: 0125036. ISSN: 0026-8925.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199807

ENTRY DATE: Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980720

AB In previous papers we have reported the characterisation of mitochondrial mutator mutants of *Schizosaccharomyces pombe*. In contrast to nuclear mutator mutants known from other eucaryotes, this mutator phenotype correlates with mutations in an unassigned open reading frame (urf a) in the mitochondrial genome. Since an efficient biolistic transformation system for fission yeast mitochondria is not yet available, we relocated the mitochondrial urf a gene to the nucleus. As host strain for the ectopic expression, we used the nonsense mutant ana(r)-6, which carries a premature stop codon in the urf a gene. The phenotype of this mutant is characterised by continuous segregation of progeny giving rise to fully respiration competent colonies, colonies that show moderate growth on glycerol and a fraction of colonies that are unable to grow on glycerol. The phenotype of this mutant provides an excellent tool with which to study the effects on the mutator phenotype of ectopic expression of the urf a gene. Since a UGA codon encoding tryptophan is present in the original mitochondrial gene, we constructed two types of expression cassettes containing either the mitochondrial version of the urf a gene (mt-urf a) or a standard genetic code version (nc-urf a; UGA replaced by UGG) fused to the N-terminal import leader sequence of the cox4 gene of *Saccharomyces cerevisiae*. We show that the expression of the mt-urf a gene in its new location is able to cure, at least in part, the phenotype of mutant ana(r)-6, whereas the expression of the nc-urf a gene completely restores the wild-type (non-mutator) phenotype. The significant similarity of the urf a gene to the mitochondrial var1 gene of *S. cerevisiae* and homologous genes in other yeasts suggests that the urf a gene product might be a ribosomal protein with a dual function in protein synthesis and maintenance of mitochondrial DNA integrity.

L10 ANSWER 5 OF 9 MEDLINE

ACCESSION NUMBER: 1998173057 MEDLINE

DOCUMENT NUMBER: 98173057 PubMed ID: 9514124

TITLE: High-level expression of bovine beta-lactoglobulin in *Pichia pastoris* and characterization of its physical properties.

AUTHOR: Kim T R; Goto Y; Hirota N; Kuwata K; Denton H; Wu S Y; Sawyer L; Batt C A

CORPORATE SOURCE: Department of Food Science, Cornell University, Ithaca, NY 14853, USA.

SOURCE: PROTEIN ENGINEERING, (1997 Nov) 10 (11) 1339-45.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980507
Last Updated on STN: 19980507
Entered Medline: 19980424

AB Bovine beta-lactoglobulin (BLG) variant A has been expressed in the methylotropic yeast *Pichia pastoris* by fusion of the cDNA to the sequence coding for the alpha-mating factor prepro-leader peptide from *Saccharomyces cerevisiae*. *P. pastoris* Mut+ transformants were obtained by single cross-over integration of the BLG-containing vector into the AOX1 locus. In a fed-batch fermenter, a cell density of approximately 300 mg/ml was achieved by controlled glycerol feeding for a total of 24 h. After 72 h of methanol induction, the secreted BLG reached levels of > 1 g/l. The secreted protein could be purified to homogeneity by ion-exchange chromatography. Amino-terminal sequencing of the secreted BLG revealed that the Glu-Ala spacer repeats inserted between the mature protein and the alpha-factor prepro-leader were still present. The purified protein was characterized by a number of methods, including CD spectroscopy, guanidine-HCl unfolding, crystallization and two-dimensional 1H-NMR spectroscopy. By all of these measures, the physical characteristics of recombinant BLG were indistinguishable from those of the native purified bovine BLG, making it useful as a model for protein folding and other biophysical studies.

L10 ANSWER 6 OF 9 MEDLINE
ACCESSION NUMBER: 1998052158 MEDLINE
DOCUMENT NUMBER: 98052158 PubMed ID: 9390456
TITLE: Biosynthetic production of type II fish antifreeze protein: fermentation by *Pichia pastoris*.
AUTHOR: Loewen M C; Liu X; Davies P L; Daugulis A J
CORPORATE SOURCE: Department of Biochemistry, Queen's University, Kingston, Ontario, Canada.
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1997 Oct) 48 (4) 480-6.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 19980206
Entered Medline: 19980127

AB Sea raven type II antifreeze protein (SRAFP) is one of three different fish antifreeze proteins isolated to date. These proteins are known to bind to the surface of ice and inhibit its growth. To solve the three-dimensional structure of SRAFP, study its ice-binding mechanism, and as a basis for engineering these molecules, an efficient system for its biosynthetic production was developed. Several different expression systems have been tested including baculovirus, *Escherichia coli* and yeast. The latter, using the methylotrophic organism *Pichia pastoris* as

the host, was the most productive. In shake-flask cultures the levels of SRAFP secreted from *Pichia* were up to 5 mg/l. The recombinant protein has an identical activity to SRAFP from sea raven serum. In order to increase yields further, four different strategies were tested in 10-l fermentation vessels, including: (1) optimization of pH and dissolved oxygen, (2) mixed feeding of methanol and glycerol with *Mut*(s) clones, (3) supplementation of amino acid building blocks, and (4) methanol feeding with *Mut*+ clones. The mixed-feeding/*Mut*(s) strategy proved to be the most efficient with SRAFP yields reaching 30 mg/l.

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:269746 CAPLUS
DOCUMENT NUMBER: 131:71057
TITLE: Effects of glycerol concentration and pH on growth of recombinant *Pichia pastoris* yeast
AUTHOR(S): Chiruvolu, V.; Eskridge, K.; Cregg, J.; Meagher, M.
CORPORATE SOURCE: Scios, Inc., Mountain View, CA, 94043, USA
SOURCE: Applied Biochemistry and Biotechnology (1999), Volume Date 1998, 75(2-3), 163-173
CODEN: ABIBDL; ISSN: 0273-2289
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Batch fermns. were used to study the effect of different glycerol concns. and pH conditions on growth of recombinant *Pichia pastoris*. Two strains of *P. pastoris* were used: a wild-type in methanol utilization (*Mut*+) and a mutant defective in methanol utilization (*Mut*-). Under const. pH conditions of 5.0, glycerol concns. up to 12% were efficiently utilized. Cell yield (*Yx/s*) of about 0.8 and a final cell d. of about 95 g/L (dry cell) were achieved. However, there were significant differences (probability [*Pr*] > *F* 0.0351) in specific growth rates between the initial glycerol concns. of 2, 7, and 12%. When fermns. were conducted without pH control, growth continued until the pH had decreased to about 2.5. Growth stopped at pH 2.2 with uncontrolled pH, and residual glycerol concns. were greater than 2%. As a result, *Yx/s* decreased to about 0.3. There were no differences between *Mut*+ and *Mut*- strains during cell growth on glycerol.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1990:629613 CAPLUS
DOCUMENT NUMBER: 113:229613
TITLE: Fermentation development of recombinant *Pichia pastoris* expressing the heterologous gene: bovine lysozyme
AUTHOR(S): Brierley, R. A.; Bussineau, C.; Kosson, R.; Melton, A.; Siegel, R. S.
CORPORATE SOURCE: Salk Inst. Biotechnol./Ind. Associates, San Diego, CA, 92138, USA
SOURCE: Annals of the New York Academy of Sciences (1990), 589(Biochem. Eng. 6), 350-62
CODEN: ANYAA9; ISSN: 0077-8923
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant *P. pastoris* strains expressing bovine lysozyme c2 were used as a model system to develop more productive fermn. processes. Two methods for improving the productivity of recombinant *P. pastoris* fermns. were examd. The first involved the use of a high-cell-d., mixed-substrate, fed-batch fermn. The mixed-feed consisted of a repressing carbon source, glycerol, and MeOH as the inducing carbon source. Because the glycerol was fed into the fermentor at limiting amts., repression of the AOX1 promoter did not occur and the growth rate and MeOH uptake rate of the Mut- strain increased as a result of the growth on glycerol as well as MeOH during the induction phase. The optimal NL fermn., utilizing limiting glycerol and excess MeOH feeds (1:2 glycerol:MeOH), resulted in a 4.5-fold improvement in volumetric productivity of the prodn. phase. Scaleup and application of mixed feeds in continuous culture are currently being addressed. The second method involved the use of Mut+ strains in which MeOH utilization is similar to wild-type *P. pastoris*. The Mut+ phenotype allowed development of a process that increased the volumetric productivity of the induction stage by 6.5-fold. This improvement was demonstrated at the 1- and 10-L scale and at cell densities in excess of 130 g/L dry wt. The Mut+ strain also allowed the use of continuous culture for prodn. of bovine lysozyme at a volumetric productivity of 12-15 mg/L-h. An 8-L (working vol.) continuous fermn. produced 19 g of bovine lysozyme in 200 h compared to the original Mut- process, which produced 3 g of lysozyme in the same fermentor in 125 h.

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1971:72243 CAPLUS

DOCUMENT NUMBER: 74:72243

TITLE: Influence on gonadotropins by progestational agents

AUTHOR(S): Bettendorf, Gerhard

CORPORATE SOURCE: Abt. Klin. Expt. Endokrinol.,
Universitaetsfrauenklin., Hamburg-Eppendorf, Ger.

SOURCE: Bull. Schweiz. Akad. Med. Wiss. (1970),
25(4-6), 353-67

CODEN: BSAMA5

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The influences of progesterone (I) and 6-chloro-9.beta.,10.alpha.-pregna-1,4,6-triene-3,20-dione (II; Ro 4-8347) on the effect of PMS (pregnant mare serum) in the mouse uterus test (MUT) and the augmentation test (AT) and also on the effect of HCG in the ovarian ascorbic acid depletion (OAAD) test were studied. It was shown in ovariectomized mice that both I and II are antiestrogenic. Administration of 0.1 mg II inhibited the influence of 0.09 .mu.g estradiol on uterine wt. >50%. I in comparison seemed to have a stronger antiestrogenic effect. In the MUT in intact immature animals with PMS, neither I nor II (0.1 mg) influenced the increase in uterine wt. When the dosage was increased to 0.5 mg, a significantly lower increase in uterus wt. was found. With 0.5 mg the gonadotropic effect was almost completely suppressed. In the AT, inhibition occurred with the lowest dose of PMS (5 IU). With 10 IU PMS no significant effect of I was found, but different doses of II had a neg. effect on the increase of ovarian wt. With higher amts. of I no effect was seen. The OAAD test was done with 3, 6, and 12 IU HCG (human chorionic gonadotropin). The progestins were given 3 and 12 hr, resp., before the test. In the 3-hr expt. the LH effect was lowered by both progestins and with all doses used. There were no marked differences

between the 2 steroids. Only in the group with 12 IU HCG was the inhibition smaller after administration of II compared to I. When the progestins were administered 12 hr before the OAAD, test differences between I and II were found; i.e. the LH effect was not influenced by I, but II enhanced the LH effect. It is concluded that there are certain pos. effects on gonadotropin action: (1) the production or release of pituitary gonadotropins is influenced probably by altering the ratio of FSH to LH, and (2) the effect of exogenous gonadotropin also is influenced by different progestational agents. Under some conditions progestins can have a pos. influence on LH action and probably a neg. one on FSH. These findings and those of others are discussed in detail.

=> d ibib abs 1-4

L13 ANSWER 1 OF 4 MEDLINE
ACCESSION NUMBER: 1998359889 MEDLINE
DOCUMENT NUMBER: 98359889 PubMed ID: 9693060
TITLE: Production and purification of the heavy-chain fragment C of botulinum neurotoxin, serotype B, expressed in the methylotrophic yeast *Pichia pastoris*.
AUTHOR: Potter K J; Bevins M A; Vassilieva E V; Chiruvolu V R; Smith T; Smith L A; Meagher M M
CORPORATE SOURCE: Department of Food Science and Technology, Biological Process Development Facility, University of Nebraska-Lincoln, 68583-0919, USA.
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1998 Aug) 13 (3) 357-65.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980925
Last Updated on STN: 19980925
Entered Medline: 19980917

AB A recombinant Hc fragment of botulinum neurotoxin, serotype B (rBoNTB(Hc)), has been successfully expressed in a **Mut+** strain of the methylotrophic yeast *Pichia pastoris* for use as an antigen in a proposed human vaccine. The fermentation process consisted of batch phase on **glycerol**, followed by **glycerol** and methanol fed-batch phases yielding a final cell mass of 60 g/L (dcw) and was easily scaled-up to 60 L. A multistep ion-exchange chromatographic purification process was employed to **produce** 99% pure Hc fragment. The final yield of the purified antigen was 390 mg per kilogram of wet cell mass. The purified Hc fragment of serotype B was stable, elicited an immune response in mice, and protected upon challenge with native botulin. Copyright 1998 Academic Press.

L13 ANSWER 2 OF 4 MEDLINE
ACCESSION NUMBER: 1998052158 MEDLINE
DOCUMENT NUMBER: 98052158 PubMed ID: 9390456
TITLE: Biosynthetic production of type II fish antifreeze protein: fermentation by *Pichia pastoris*.
AUTHOR: Loewen M C; Liu X; Davies P L; Daugulis A J
CORPORATE SOURCE: Department of Biochemistry, Queen's University, Kingston, Ontario, Canada.
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1997 Oct) 48 (4) 480-6.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 19980206

Entered Medline: 19980127

AB Sea raven type II antifreeze protein (SRAFP) is one of three different fish antifreeze proteins isolated to date. These proteins are known to bind to the surface of ice and inhibit its growth. To solve the three-dimensional structure of SRAFP, study its ice-binding mechanism, and as a basis for engineering these molecules, an efficient system for its biosynthetic production was developed. Several different expression systems have been tested including baculovirus, *Escherichia coli* and yeast. The latter, using the methylotrophic organism *Pichia pastoris* as the host, was the most productive. In shake-flask cultures the levels of SRAFP secreted from *Pichia* were up to 5 mg/l. The recombinant protein has an identical activity to SRAFP from sea raven serum. In order to increase yields further, four different strategies were tested in 10-l fermentation vessels, including: (1) optimization of pH and dissolved oxygen, (2) mixed feeding of methanol and glycerol with Mut(s) clones, (3) supplementation of amino acid building blocks, and (4) methanol feeding with Mut+ clones. The mixed-feeding/Mut(s) strategy proved to be the most efficient with SRAFP yields reaching 30 mg/l.

L13 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:645874 CAPLUS

DOCUMENT NUMBER: 127:306642

TITLE: Recombinant protein production in an alcohol oxidase-defective strain of *Pichia pastoris* in fedbatch fermentations

AUTHOR(S): Chiruvolu, Vijay; Cregg, James M.; Meagher, Michael M.

CORPORATE SOURCE: Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE, 68583-0708, USA

SOURCE: Enzyme and Microbial Technology (1997), 21(4), 277-283

CODEN: EMTED2; ISSN: 0141-0229

PUBLISHER: Elsevier

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The methylotrophic yeast *Pichia pastoris* synthesizes high levels of alc. oxidase from the AOX1 gene during growth on methanol as a carbon source. The authors used a transcriptional fusion of the lacZ gene to the AOX1 promoter as a model system for investigating recombinant protein prodn. in an alc. oxidase (aox1, aox2) defective strain. Growth and recombinant protein prodn. with glycerol as the carbon source (fed at various const. feedrates) was studied. A feedrate of 1 g L⁻¹ h⁻¹ was found to be optimum, resulting in a specific activity of 8.62 .times. 10⁴ U mg⁻¹ dry cell. The specific yield did not improve when glycerol was increased in steps. High feeding rates gave low specific yields (U mg⁻¹ dry cell mass) and high cell masses. Low protein yields at higher glycerol feedrates were due to partial repression of the AOX1 promoter by glycerol and the byproduct, ethanol. In comparison, the wild type (Mut+) strain gave a max. specific yield of 5.52 .times. 10⁴ U mg⁻¹ dry cell.

L13 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1990:629613 CAPLUS

DOCUMENT NUMBER: 113:229613

TITLE: Fermentation development of recombinant *Pichia*

pastoris expressing the heterologous gene: bovine lysozyme

AUTHOR(S): Brierley, R. A.; Bussineau, C.; Kosson, R.; Melton, A.; Siegel, R. S.

CORPORATE SOURCE: Salk Inst. Biotechnol./Ind. Associates, San Diego, CA, 92138, USA

SOURCE: Annals of the New York Academy of Sciences (1990), 589(Biochem. Eng. 6), 350-62
CODEN: ANYAA9; ISSN: 0077-8923

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Recombinant *P. pastoris* strains expressing bovine lysozyme c2 were used as a model system to develop more productive fermn. processes. Two methods for improving the productivity of recombinant *P. pastoris* fermns. were examd. The first involved the use of a high-cell-d., mixed-substrate, fed-batch fermn. The mixed-feed consisted of a repressing carbon source, glycerol, and MeOH as the inducing carbon source. Because the glycerol was fed into the fermentor at limiting amts., repression of the AOX1 promoter did not occur and the growth rate and MeOH uptake rate of the **Mut-** strain increased as a result of the growth on glycerol as well as MeOH during the induction phase. The optimal NL fermn., utilizing limiting glycerol and excess MeOH feeds (1:2 glycerol:MeOH), resulted in a 4.5-fold improvement in volumetric productivity of the **prodn.** phase. Scaleup and application of mixed feeds in continuous culture are currently being addressed. The second method involved the use of **Mut+** strains in which MeOH utilization is similar to wild-type *P. pastoris*. The **Mut+** phenotype allowed development of a process that increased the volumetric productivity of the induction stage by 6.5-fold. This improvement was demonstrated at the 1- and 10-L scale and at cell densities in excess of 130 g/L dry wt. The **Mut+** strain also allowed the use of continuous culture for prodn. of bovine lysozyme at a volumetric productivity of 12-15 mg/L-h. An 8-L (working vol.) continuous fermn. produced 19 g of bovine lysozyme in 200 h compared to the original **Mut-** process, which produced 3 g of lysozyme in the same fermentor in 125 h.